GENETIC DIFFERENTIATION BETWEEN SCHISTOSOMA MEKONGI AND S. JAPONICUM: AN ELECTROPHORETIC STUDY

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INTRODUCTION

The detection of enzyme variation by electrophoresis provides a convenient basis for the genetic characterization of populations and species, and for the assessment of the degree of differentiation between such groups. By revealing the products of structural genes, the electrophoretic method provides an estimate of phylogenetic affinity that is unattainable by more conventional taxonomic methods. Using this technique, we are able to add an important dimension to the characterization of Schistosoma mekongi, a newly-described species which is morphologically very similar to S. japonicum (Davis et al., 1976; Voge et al., 1978). Specifically, we demonstrate that S. mekongi is genetically distinct from S. japonicum and that the divergence found between the two species is greater than that between various geographic strains of S. japonicum.

The application of the techniques of gel electrophoresis of enzymes to systematic and evolutionary problems is now well established. The reader unfamiliar with the development of this field is referred to extensive reviews by Avise (1974, 1976), Lewontin (1974), Ayala (1975), Nei (1975), and Selander (1976). Surprisingly, these powerful techniques have not been applied to the genus Schistosoma; previous electrophoretic studies have involved pooled samples of worms and genetic interpretations have thus been precluded (e.g., Conde-del-Pino et al., 1966, 1968; Yoshimura, 1968; Oya et al., 1970; Coles, 1970, 1971a,b; Ruff et al., 1971, 1973; Ross, 1976). In contrast, the present study is based on samples derived from individual schistosomes. The separate allelic variants segregating at a given locus can thus be detected on the basis of differences in mobility in the gel, and intra- and inter-population variation can be assessed.

Previous work on Schistosoma japonicum (Ruff et al., 1973) demonstrated differences between the Japanese, Philippine and Formosan strains based on polyacrylamide disc gel electrophoresis of total soluble proteins. The authors concluded that the Japanese and Formosan strains were more closely related to each other than to the Philippine strain. The present study involves a horizontal starch gel technique, which presents definite advantages over acrylamide for surveys of genetic variation. For instance, up to 20 samples may be electrophoresed on a

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satisfactory separation of a wider range of enzyme systems (Brewer, 1970). The use of stains for specific enzyme systems rather than for total soluble proteins enables a more accurate estimate of the number of loci involved in the comparisons. The sample of loci surveyed in the present study was moderate, but representative of a wide range of enzymatic functions.

MATERIALS AND METHODS

Schistosoma mekongi was characterized on the basis of a strain isolated from Khong Island, Laos, in 1971. It was compared with four geographic strains of S. japonicum. The four strains were isolated, respectively, from China in 1928, from Pang-Hu, Formosa, in 1962, from Kofu province, Japan, in 1968, and from Pulo, Leyte, Philippines, in 1969. A strain of S. mansoni isolated from Puerto Rico in 1969 (NIH-Sm-PR-1) served as a reference. The strains of S. mekongi and S. japonicum were obtained from the Center for Tropical Diseases, University of Lowell, Massachusetts, U.S.A. The S. mansoni strain was originally obtained from Dr. C.S. Richards, U.S. National Institutes of Health, and is maintained at the Department of Biological Sciences, Purdue University, Indiana, U.S.A., where the present study was conducted. S. mekongi and S. japonicum samples were derived by infecting female mice with 25-50 cercariae from a pool of 5-54 shedding snails. S. mansoni samples were derived by infecting female mice with 150-200 cercaria from a pool of 10-25 shedding snails.

Infected mice were killed by injection with sodium pentobarbital and heparin, 34-77 days postinfection, and sexually mature schistosomes were recovered by perfusion and dissection. Schistosomes were rinsed three times in Eagle's Minimal Essential Medium with Earle's salts (Grand Island Biological Co., New York, U.S.A.). Individual motile worms were then crushed with a ground glass stopper on a glass slide in approximately 10 µl of distilled water at 4°C. The resulting homogenate was immediately absorbed on a 9.5 x 3.5 mm tab of filter paper (Whatman No. 3), which was then inserted along a transverse cut made in a cold starch gel.

Horizontal electrophoresis was performed on 12.5% starch gels (Electrostarch Co., Madison, Wisconsin, U.S.A.) at 4°C, following the techniques of Selander et al. (1971). The following buffer systems were employed: 1) continuous tris-citrate, pH 6.7 (Selander et al., 1971); 2) histidine, pH 7.0 (Brewer, 1970); 3) tris-glycine, pH 8.3, consisting of 0.005M tris, 0.025M glycine for the gel, and 0.3M borate, pH 8.0 for the electrode buffer (Dr. Morris Levy, Purdue University, pers. comm.). Following electrophoresis, each gel was cut into four horizontal slices, 2 mm thick, and stained for various enzyme systems. Enzyme staining techniques followed Brewer (1970), Shaw & Prasad (1970) and Selander et al. (1971). The gel slices were incubated at 37°C until the enzyme patterns developed, then were rinsed and fixed in a 5:5:1 solution of water, methanol, and glacial acetic acid. Enzyme activity patterns were recorded and photographed immediately.

For greater accuracy, comparisons between strains and species were only made from worms electrophoresed on the same gel. Occasionally, samples of whole mouse blood were also electrophoresed to check for the possible presence of host proteins in the schistosome homogenates. Bands of enzyme activity, or electromorphs (King & Ohta, 1975), were characterized by the distance they migrated on the gel, relative to the migration distance of the corresponding electromorph of Schistosoma mansoni controls run on the same gel. For example, under the specified conditions, the aldolase electromorph in S. mekongi migrated 1.9 times as far as the S. mansoni electromorph, which was given a mobility of 100; it was accordingly designated as Ald. The relative mobility values thus obtained are approximations, and only serve to designate different electrophoretic variants of one enzyme. The exact determination of a protein's electrophoretic mobility requires the use of more elaborate methods (Johnson, 1977) and is not directly relevant to the purposes of this study. No attempt was made to correlate distance between electromorphs of two strains with the degree of genetic differentiation between them, since there is no simple relationship between a genetic change, such as a single amino acid substitution, and a change in relative mobility. In fact, many amino acid substitutions are not even detectable electrophoretically. We used NIH-Sm-PR-1 as a control, because we have found this strain to be invariant at 18 loci (Fletcher et al., in prep.), and because it is readily accessible to workers in the U.S. For most enzymes studied, we were able to examine the following number of adult male and female schistosomes: S. mekongi (males, 19; females, 7); S. japonicum (males, 19; females, 7); S. mansoni (males, 3; females, 4); Formosa (32; 8); China (19; 5). Except where noted, all comparative statements refer to male worms.
Comparisons between strains were based on the proportion of loci that differed in electrophoretic mobility rather than directly on the proportion of differing electromorphs, since a particular locus may express more than one band of activity. Because none of the strains included in the present study showed any intrastrain electrophoretic variation, the genetic interpretations we made are tentative, and are extrapolated from patterns of variation found in other strains of *Schistosoma mansoni* (Fletcher et al., in prep.). For example, in some strains of *S. mansoni*, the anodal band pattern for malate dehydrogenase shows variation while the cathodal band pattern does not. We may thus assume that two enzyme loci are involved, Mdh-1 migrating anodally and Mdh-2 migrating cathodally.

**RESULTS**

Consistent and interpretable patterns were obtained with ten enzyme systems, representing a conservative estimate of 13 loci. All electromorphs were distinct from those of host proteins. Each strain surveyed was invariant at the loci examined. *Schistosoma mekongi* clearly differed in electrophoretic mobility from *S. japonicum* in at least 82% of the loci (Table 1). The actual difference varied between 82% (nine of 11 loci) for the Chinese and Formosan strains, 83% (10 of 12 loci) for the Japanese strain, and 91% (10 of 11 loci) for the Philippine strain. No significance is attached to these differences in the estimate of interspecific differentiation in view of the small number of genes sampled. In contrast, differences between the four geographic strains of *S. japonicum* affected only 17 to 36% of the loci (two of 12 to four of 11 loci). Both species of Asian schistosomes diverged from *S. mansoni* at 92 to 100% of the loci (12 of 13 to 12 of 12 loci).

There were consistent differences in enzyme activity (as detected by stain intensity) and in mobility between male and female schistosomes of a given species. The specific differences found are described below, but not commented on, as they are of peripheral interest to the present study. The adult worms compared were not always of equal age (time after infection of the mouse host), but no age-related variation in enzyme mobility was detected. The following are descriptions of the electrophoretic phenotypes and presumed genotypes of the various schistosome strains examined.

**Phosphoglucomutase (E.C. 2.7.5.1)**

Two loci were detected on tris-glycine gels. *Pgm-1* appeared as a two-banded pattern of identical mobility in male and female worms (Figs. 1, 3). The mobility of this doublet was species-specific (Table 1). The Chinese strain of *Schistosoma japonicum* differed slightly from the other three strains in the mobility of the doublet. A faint slower band found in *S. mansoni* was considered to represent a second locus, *Pgm-2*, because it showed allelic variation in a recently-isolated strain of *S. mansoni* from Egypt (Fletcher et al., in prep.). In that strain, individual worms exhibit either a slow band, a fast band, or a double-banded heterozygote. In NIH-Sm-PR-1, *Pgm-2* is fixed for the slow band. In *S. mekongi* and *S. japonicum*, this locus, if expressed, has not yet been identified.

**Aldolase (E.C. 4.1.2.13)**

On tris-glycine gels, a single well-defined band of enzyme activity was resolved for male and female worms. The three species were characterized by electromorphs of different mobility (Figs. 1, 4; Table 1). There was variation between the *Schistosoma japonicum* strains, with the electromorph in the Philippine and Japanese strains migrating slightly further than that in the Chinese and Formosan strains. Female schistosomes stained less intensely than did the males, presumably due to their smaller size, although intrinsic differences between the sexes have been noted for other enzymes (Coles, 1973). In female *S. mekongi* and *S. japonicum*, the electromorphs migrated at slightly different rates than those in the males. No differences in mobility were found between male and female *S. mansoni*. 
ELECTROPHORESIS OF SCHISTOSOMA

FIG. 1. Schematic diagram of variation in electromorph patterns of individual adult male schistosomes. Patterns shown are those resolved on tris-glycine gels after 2.5 hrs at 250 V. Various grades of shading indicate differences in stain intensity. See Table 1 for relative mobilities.

Enzyme system abbreviations: PGM = phosphoglucomutase; ALD = aldolase; G6PD = glucose-6-phosphate dehydrogenase; PGI = phosphoglucoisomerase; GDH = glutamate dehydrogenase.

Schistosome strain abbreviations: M = Schistosoma mekongi; J = S. japonicum - all strains; p, j, f, c = S. japonicum - Philippines, Japan, Formosa and China, respectively; m = S. mansoni.

Glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49)

A single band of activity was resolved in male worms on tris-glycine gels (Fig. 1). The mobility of the Schistosoma mekongi electromorph was considered identical to that of the electromorphs of S. japonicum from Japan, Formosa and China (Table 1). The electromorphs of S. japonicum from the Philippines and S. mansoni from Puerto Rico each differed distinctly in mobility. Female worms of all three species showed a band identical in mobility to that found in the males, but of lesser stain intensity. In individual female S. mekongi and S. japonicum and in pooled samples of the smaller S. mansoni females, an additional band migrating at a faster rate was also present. This faster band was similar in mobility to an electromorph present in mouse blood samples, so it may be of host origin.

Phosphoglucoisomerase (E.C. 5.3.1.9)

On tris-glycine gels, a single band of activity was resolved for male and female worms. Schistosoma mekongi showed a band of slower mobility than S. japonicum, while differences were also apparent between S. japonicum strains (Fig. 1, Table 1). An additional cathodal electromorph in the Japanese strain was considered to represent a second locus. After more prolonged staining, the first set of bands faded and another system of bands, independent from the first, appeared. This second system, possibly representing another enzyme, is not included in the present survey due to insufficient data.

Glutamate dehydrogenase (E.C. 1.4.1.3)

A faint but well-defined band was resolved after staining tris-glycine gels overnight. Schistosoma mekongi and S. japonicum migrated the same distance and S. mansoni migrated slightly further (Fig. 1, Table 1). Females of the two Asian species migrated a little faster and stained more intensely than did the male worms.

Hexokinase (E.C. 2.7.1.1)

Both on tris-citrate and histidine gels, Schistosoma mekongi and S. japonicum males were
characterized by a single band of different width and relative mobility, while *S. mansoni* males showed a two-banded pattern (Fig. 2, Table 1). No variation was detected between *S. japonicum* strains. Female worms of all three species were characterized by a single band, identical in mobility to that of male worms (in *S. mansoni*, it was identical to the slower male electromorph). In *S. mekongi* and *S. japonicum*, a few individuals, male and female, showed an additional slow band. More study is needed to determine whether these variants are due to artifacts in sample preparation or reflect actual genetic heterogeneity. Proteolytic degradation has been incriminated as a source of heterogeneity in yeast hexokinase (Gazith et al., 1968).

Malate dehydrogenase (E.C. 1.1.1.37)

Evidence for two loci was detected both on tris-citrate and histidine gels (Fig. 2, Table 1). *Mdh-1* migrated as a single band toward the anode, with different mobilities observed for the three species. No variation was detected between *Schistosoma japonicum* strains. Female worms of all three species were characterized by a single band, identical in mobility to that of male worms (in *S. mansoni*, it was identical to the slower male electromorph). In *S. mekongi* electromorph showed signs of degradation, usually appearing as a streak migrating anodally rather than a well-defined band. *Mdh-2* was poorly resolved under all conditions tested; it migrated cathodally as a smear close to the origin for *S. japonicum* and *S. mansoni*, with no distinct interspecific differences, and was usually absent for *S. mekongi*. Male and female worms showed identical electrophoretic mobility at both loci.

Acid phosphatase (E.C. 3.1.3.2)

Interspecific variation was detected both on tris-citrate and histidine gels (Figs. 2, 5; Table 1). For both male and female worms, a single well-defined band migrated cathodally in *Schistosoma mekongi* and anodally in *S. japonicum*. Again, no variation was detected between *S. japonicum* strains. *S. mansoni* worms showed a streak remaining at the origin rather than a well-defined band. Extensive smearing extending below the band of activity to the origin occurred under the specified conditions, especially for *S. japonicum*, and suggests the presence of other electromorphs, or partial degradation of the enzyme.

Lactate dehydrogenase (E.C. 1.1.1.27)

Both on tris-citrate and histidine gels, enzyme activity was detected only in male schistosomes, presumably because enzyme activity in females is too low to be detected by the methods.
TABLE 1. Mobility of various enzyme electromorphs in male Schistosoma mekongi and S. japonicum, expressed relative to that of the corresponding electromorph in S. mansoni. Electromorph mobility was measured from the lower margin of the band of activity. Cathodally-migrating electromorphs are designated by a negative sign preceding the relative mobility value. Enzymes are abbreviated as in text.

<table>
<thead>
<tr>
<th>Enzyme locus</th>
<th>S. mekongi</th>
<th>S. japonicum</th>
<th>S. mansoni</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Philippine</td>
<td>Japanese</td>
<td>Formosan</td>
</tr>
<tr>
<td>Pgm-1</td>
<td>108</td>
<td>89</td>
<td>89</td>
</tr>
<tr>
<td>Pgm-2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ald</td>
<td>190</td>
<td>270</td>
<td>270</td>
</tr>
<tr>
<td>G6pd</td>
<td>85</td>
<td>90</td>
<td>85</td>
</tr>
<tr>
<td>Pgi-1</td>
<td>200</td>
<td>333</td>
<td>333</td>
</tr>
<tr>
<td>Pgi-2</td>
<td>-</td>
<td>-</td>
<td>-400</td>
</tr>
<tr>
<td>Gdh</td>
<td>97</td>
<td>97</td>
<td>97</td>
</tr>
<tr>
<td>Hx</td>
<td>200</td>
<td>225</td>
<td>225</td>
</tr>
<tr>
<td>Mdh-1</td>
<td>110</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>Mdh-2</td>
<td>-</td>
<td>-100</td>
<td>-100</td>
</tr>
<tr>
<td>Acp</td>
<td>-250</td>
<td>1700</td>
<td>1700</td>
</tr>
<tr>
<td>Ldh</td>
<td>-50</td>
<td>1300</td>
<td>1300</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>700</td>
<td>700</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ga3pd</td>
<td>150</td>
<td>133</td>
<td>133</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>

* = absence of any activity at a locus identified in congeners; ** = absence of second or third band of activity.

used. Patterns for Schistosoma japonicum were not consistently well resolved, possibly due to lower stability of the enzyme in that species. Optimal resolution of S. japonicum and S. mansoni gave three-banded anodal patterns of different mobilities for each species. For S. mekongi, the enzyme migrated cathodally as an intensely-staining band close to the origin (Fig. 2, Table 1).

Glyceraldehyde-3-phosphate dehydrogenase (E.C. 1.2.1.12)

This enzyme was partially degraded under all conditions tested, and zones of activity were
FIG. 3. Phosphoglucomutase patterns for adult male schistosomes as resolved on tris-glycine gels after 2.5 hrs at 250 V. First position: *Schistosoma mansoni*; second and third positions: *S. mekongi*; fourth and fifth positions: *S. japonicum*, Philippine strains. FIG. 4. Aldolase patterns for adult male schistosomes as resolved on tris-glycine gels after 2.5 hrs at 250 V. Positions as in Fig. 3. FIG. 5. Acid phosphatase patterns for adult male schistosomes as resolved on histidine gels after 3 hrs at 80 V. Positions as in Fig. 3.

Smeared. *Schistosoma mekongi*, however, did show a pattern distinct from that seen in *S. japonicum* and *S. mansoni*. Again, no variation was detected within *S. japonicum* (Fig. 2, Table 1).

**DISCUSSION**

The results presented here provide strong evidence for the species status of *Schistosoma mekongi*. *S. mekongi* is genetically distinct from *S. japonicum* at between 82 and 91% of the 11-12 loci involved in the comparison, while differences between *S. japonicum* strains affect only 17 to 36% of the same loci. Using these data, Nei's (1972) indices of genetic identity \( I \) and distance \( D \) were calculated for all pairwise comparisons between strains of *S. mekongi*, *S. japonicum* and *S. mansoni* (Table 2). Nei's \( I \) is defined as the normalized identity of genes between two populations and \( D \) as a minimum estimate of the average number of codon differences per locus between the two populations. Because no genetic variation was found for any of the strains examined, the formulae for \( I \) and \( D \) between any two strains are reduced to:

\[
I = 1 - c \\
D = -\log I
\]

\( c \) being the proportion of loci that differ in electrophoretic mobility. The genetic distances between *S. mekongi* and each strain of *S. japonicum*, 1.71-2.40, fall in the upper range of those reported for congeneric animal species (the range being 0.05-2.80 (Nei, 1975)).

The taxonomic status of the geographic strains of *Schistosoma japonicum*, as well as the phylogenetic affinities between them, have been the subject of much debate (Hsü & Hsü, 1962; Davis, 1970). Although the genetic distances among the four laboratory strains are typical of those found among subspecies in other taxa, it would be premature to employ our data for formal phylogenetic purposes. The genetic distances given in Table 2 are based on a limited number of loci and so must be considered as preliminary estimates. For example, the \( D \) value of \( \infty \) between *S. mekongi* and *S. mansoni* is surely due to sampling error. Thus the sample of loci examined should be increased, but, even more important, additional field-collected populations of *S. japonicum* from each geographic area should be surveyed.
TABLE 2. Nei's (1972) genetic identity (I), above diagonal, and genetic distance (D), below diagonal, between various strains of Schistosoma, based on available electrophoretic data (Table 1) for 11-13 loci. Strains abbreviated as in Fig. 1.

<table>
<thead>
<tr>
<th>Schistosome</th>
<th>M</th>
<th>p</th>
<th>j</th>
<th>f</th>
<th>c</th>
<th>m</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. mekongi (M)</td>
<td>0.000</td>
<td>0.091</td>
<td>0.167</td>
<td>0.182</td>
<td>0.182</td>
<td>0.000</td>
</tr>
<tr>
<td>S. japonicum</td>
<td>2.398</td>
<td>0.000</td>
<td>0.833</td>
<td>0.818</td>
<td>0.636</td>
<td>0.083</td>
</tr>
<tr>
<td>Philippines (p)</td>
<td>1.792</td>
<td>0.182</td>
<td>0.000</td>
<td>0.833</td>
<td>0.667</td>
<td>0.077</td>
</tr>
<tr>
<td>Japan (j)</td>
<td>1.705</td>
<td>0.201</td>
<td>0.182</td>
<td>0.000</td>
<td>0.818</td>
<td>0.083</td>
</tr>
<tr>
<td>Formosa (f)</td>
<td>1.705</td>
<td>0.452</td>
<td>0.405</td>
<td>0.201</td>
<td>0.000</td>
<td>0.083</td>
</tr>
<tr>
<td>China (c)</td>
<td>1.705</td>
<td>0.452</td>
<td>0.405</td>
<td>0.201</td>
<td>0.000</td>
<td>0.083</td>
</tr>
<tr>
<td>S. mansoni (m)</td>
<td>2.485</td>
<td>2.485</td>
<td>2.485</td>
<td>2.485</td>
<td>2.485</td>
<td>2.485</td>
</tr>
</tbody>
</table>

A rough estimate of divergence time between Schistosoma mekongi and S. japonicum may be obtained from the electrophoretic data generated in this study using Nei's (1975) formula: \[ t = 5 \times 10^6 D^2 \], where \( t \) is time in years. Based on this relationship, the various strains of S. japonicum have been evolving separately from one another for 1-2 million years. S. japonicum and S. mekongi diverged from one another 8.5-12 million years ago, and the S. japonicum stock separated from the S. mansoni stock 12.5-13 million years ago. This relationship is based on certain assumptions about the constancy of mutation rates across different loci, and Nei (1975) should be consulted for a discussion of the validity of this method. The figure of approximately 10 million years for the divergence time of the S. japonicum and S. mekongi stocks is of particular interest in that it is remarkably close to the estimate arrived at by Davis (1980) on the basis of paleogeographical considerations.

In the present studies, intrastrain genetic variation within each of the schistosome strains examined was not detected. It is possible that individual variation will be detected when more enzyme systems are surveyed and when more refined techniques of enzymology are employed. For example, separation at different pH and gel concentrations and thermostability studies have uncovered a surprising amount of cryptic variation (Johnson, 1977; Throckmorton, 1977). However, in comparison to similar studies on other animal species (reviewed by Powell, 1975; Selander, 1976; Nevo, 1978), the lack of genetic variation in the schistosome strains examined is remarkable. For instance, in 93 other invertebrates, the mean proportion of polymorphic loci per population (P) was 0.397 ± 0.201, and the average proportion of heterozygotes per locus per individual (H) was 0.1123 ± 0.0720 (Nevo, 1978). Using comparable techniques, the present study obtained \( P = 0.000 \) and \( H = 0.000 \) for Schistosoma mekongi, S. japonicum (four strains), and S. mansoni (NIH-Sm-PR-1). It should be noted that all these strains have been maintained in the laboratory for periods ranging from eight to 51 years. The results obtained thus probably reflect inbreeding and selection due to laboratory conditions, as well as small original founding populations (the founder principle (Mayr, 1970)). Some support for this conclusion is emerging from ongoing studies on recent schistosome isolates (Fletcher et al., in prep.). For example, a freshly-collected sample of S. mansoni from Qalyub, Egypt, shows allelic variation at Pgm-2, and variation was found at Mdh-1, Ldh, G6pd, and a peptidase, in newly-isolated S. mansoni strains from Puerto Rico. This indicates that most of the laboratory strains of Schistosoma presently available may be atypical of natural populations. This finding has important consequences for some areas of schistosome research, such as chemotherapy or systematics. Future taxonomic work on the various geographic strains of S. japonicum should be based on recent isolates, of sufficient size and geographic diversity to adequately represent each strain.
SUMMARY

The taxonomic status of *Schistosoma mekongi* was investigated by comparing it to four strains of *S. japonicum*, originally collected in China, Formosa, Japan and the Philippines, using starch gel electrophoresis of 10 enzyme systems, representing an estimated 13 loci. The enzymes were lactate, malate, glucose-6-phosphate, glyceraldehyde-3-phosphate and glutamate dehydrogenases, hexokinase, phosphoglucomutase, acid phosphatase, aldolase and phosphoglucoisomerase. Electromorphs were characterized by their electrophoretic mobility relative to that of an invariant strain of *S. mansoni* (NIH-Sm-PR-1). Each of the schistosome strains examined was invariant at the loci surveyed. *S. mekongi* and *S. japonicum* diverged in electrophoretic mobility at 82 to 91% of the loci examined, while the *S. japonicum* strains differed from each other at 17 to 36% of the same loci. These data provide strong evidence for the species status of *S. mekongi*, Nei's genetic distance between *S. mekongi* and *S. japonicum* is estimated at 1.71-2.40, suggesting that these species have been diverging for 8.5-12 million years. The absence of individual variation was attributed to the founder effect and prolonged laboratory maintenance of the strains examined. Future taxonomic work on schistosomes should be based on recently-isolated samples that more adequately represent natural populations.

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LITERATURE CITED


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